Susceptibility of Great Wax Moth (*Galleria mellonella*) to Lufenuron insecticide and Its Toxicity to The Non- Target Organisms *Apanteles galleriae* and *Apis mellifera*

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Abstract: The greater wax moth, *Calleria mellonella* L., is one of the most threat pests of beekeeping in Egypt and worldwide. Nonetheless, methods to control such insect are limited as many safety and health challenges should be considered during planning its control program. On the one hand, *Apanteles galleriae* Wilk is widespread natural enemy of *G. mellonella*. On the other hand, the IGR insecticide lufenuron was reported to be effective against lepidopteran pests and possess a slight or no injures to honey bees. This study aimed to determine and compare the toxicity of lufenuron, and its sub-lethal concentrations against *G. mellonella*, *Apis mellifera* and *A. galleriae*. To achieve this goal, mixed with food bioassay technique was performed on *G. mellonella* larvae*, A. mellifera* workers *and A. galleriae* adults*.* The data revealed obvious toxicity of lufenuron towards *G. mellonella* larvae with LC_{50} value of 87.52, 74.86, 49.53 ppm after 24, 48 and 72 hours of treatment, respectively. In contrast, low susceptibility to lufenuron was recorded with either *A. mellifera* or *A. galleriae* with LC_{50} values 2514.38 and 2695.52 ppm after 24h of exposure, respectively. Moreover, the selectivity ratios (SR) of lufenuron according to the LC_{50} values referred to LC_{50} value of *G. mellonella* were 30.80 and 28.73, for *A. mellifera* and *A. galleriae,* respectively after 24 hours of treatment. Furthermore, the sub-lethal concentrations of lufenuron showed no toxicity against both of *A. mellifera* and *A. galleriae* up to three weeks after treatment. While 72.67, 98.46 and 100% mortalities of *G. mellonella* larvae were recorded after 1, 2 and 3 weeks following the treatment with LC_{50} (49.53) ppm of lufenuron. So, lufenuron could be utilized as a quite specific insecticide for *G. mellonella* control IPM program with no or low hazard to the natural enemy *A. galleria* as well as *A. mellifera*. This study underlines the dual effects of the biological control and IGRs as a promising approach for *G. mellonella* control in particularly during the periods of bee colonies weakness.

Keywords: *Galleria mellonella*, lufenuron, *Apanteles galleriae*, *Apis mellifera*, Honey bee pests

1.Introduction

The greater wax moth, *Calleria mellonella* L., is one of the most threat pests of beekeeping in Egypt and worldwide. It attacks wax combs inside apiaries and their surrounds causing sever damages. Seasonal most extreme damage in Egypt occurs subsequently of depositing old combs and cells with large quantities by the end of honey harvesting **(Hegazi *et al*., 2017)**. Accordingly, wax moth usually destroyed the extra drone combs, that mostly appropriate for drone production and honey storage, when not reused or properly stored **(Kwadha *et al*., 2017)**. Female moths lay their eggs on wax combs both inside or outside the hives. The hatched larvae feed on honey combes, mainly the honey bee brood combs, consuming, honey, pollen and propolis in addition to the honeybee larvae pupae and exuviae **(Paddock, 1918)**. Moreover, during feeding the wax moth larvae assembly a network of tunnels lined with silk inside the comb resultant honey to leak, starves the emerging bees and damage its structure **(Hanumanthaswamy and Rajagopal, 2017; Paddock, 1918, Kwadha *et al*., 2017)**. Consequently, wax moth attacking negatively affects the colony density and may destroy it **(Biesmeijer *et al*., 2006, Potts, *et al*., 2010)**. Also, the moths may transmit pathogens **(Charriere & Imdorf, 1999 and Kwadha *et al*., 2017)**.

Control methods of *G. mellonella* are widely diverse according to region country or season **(Sharma *et al*., 2011 and Shimanuki *et al*., 1992)** including chemical, physical and biological methods. Several chemicals are utilized to control wax moths such as; formic acid, acetic acid, sulphur dioxide, paradichlorobenzene (PDCB), naphthalene and phosphine **(Goodman *et al*., 1990, Charrière and lmdorf 1999 and Fulton, 2005)**. However, chemicals possess side effects on bee as well bee products and human health **(Charrière and Imdorf, 1997)**. The available methods to control *G. mellonella* are consider insufficient **(Burges 1978 and Shimanuki *et al*., 1992, Kwadha *et al*., 2017)**. Therefore, effective and environmentally acceptable control methods as well IPM programs are in demand.

Pollinators are vital for approximately 300,000 species of entomophilous plants around the world **(Ollerton *et al*., 2011)**. Insect pollinators are accountable for the production of around one-third of the universal human food **(Klein *et al*., 2007)** that worth over 153 billion euros worldwide **(Gallai *et al*., 2009)**. Moreover, pollination affects species diversity, ecosystem stability and food security **(FAO, 2008)**. Honey bee, *Apis mellifera* L. is the

most predominantly pollinator for crops across the world **(Garibaldi *et al*., 2013)**. Furthermore, *A. mellifera* produce many products such as; honey, royal jelly, pollen, bee bread, beeswax, bee venom and propolis that have biological actions and health benefits besides their economic importance **(Hung *et al*., 2018)**. In Egypt for several factors the value of honey production decreased within six years (2000-2016) from 25.56 million to 15.15 million USD **(FAOSTAT Database 2020)**. Assorted of pests occupy the hive surroundings such as; parasitic flies, Varroa mites, Vespa hornets, small hive beetles and wax moths leading to severe injuries. **(Abou-Shaara *et al*., 2019).**

Apanteles galleriae Wilkinson (Hymenoptera: Braconidae) a solitary koinobiont parasitoid, was originally defined by **Wilkinson (1932)** and now spread widely **(Nixon, 1965)**. *A. galleriae* is well recognised as an endoparasitoid of *G. mellonella* and existing in Egypt **(El-Hemaesy, 1983 and Hegazi *et al*., 2019)**. It attacks *G. mellonella* and *A. grisella* inside A. *mellifera* beehives **(Wani *et al*., 1994)**. The adult female of this wasp oviposits single egg within individual host larva. After hatching the parasitoid larva feeds on the host tissues until pre- pupa stage causing host death **(Galindo-Cardona *et al*., 2019)**. This parasitoid is considered the most common and efficient natural enemy of wax moths **(Gamal El-Din, 1985 and Mansour and Metwally, 2004 and Hegazy *et al*., 2019)**, thus it could be employed as a biological control for wax moths.

On the other hand, the third-generation of pesticides (insect growth regulators, IGRs) are analogues or antagonists of Juvenile Hormone interfere with insect development **(Schneiderman, 1972)** and selectively affect the physiology and development processes of insects without adverse effects on non-target organisms which suggest them as a perfect combination with biological control in IPM programs **(Dhadialla *et al*., 2005)**. A member of the third-class of IGRs is the benzoylphenyl urea derivative named lufenuron which was developed by Ciba-Geigy in 1998. Lufenuron was reported to be effective against lepidopteran pests in addition to *Bactrocera dorsalis* and *Ceratitis capitata* **(Casanã-Giner *et al*., 1999)**. Its activity against insects is contributed to collective of larvicidal, transovarial–ovicidal and ovicidal actions **(Subramanian and Shankarganesh, 2016)**. Lufenuron influential at the cellular level and inhibits chitin synthesis resulting moulting disruption. Few researches were carried out to evaluate lufenuron toxicity against *G. mellonella* but up to this study no scientific articles on lufenuron toxicity against wax moths concerned with *A. galleriae* and *A. mellifera* were found. As an approach to utilize lufenuron as environmentally acceptable insecticide to control the great wax moth; this work was carried out to investigate the toxicity of lufenuron and its sub-lethal concentrations on the larvae of the greater wax warm *G. mellonella* and its selectivity to both honey bee workers and the wax warm parasitoid *A. galleriae.*

2.Materials and methods

2.1. Tested Insects:

The great wax moth, *Galleria mellonella* (L.) Lepidoptera: Pyralidae, was originally collected from local infested hives and maintained for several generations in the laboratory of Biological control (Professor Hegazi's lab), Entomology department, Alexandria University. *G. mellonella* larvae were reared on an artificial diet that was developed by **Singh (1994)**. Meanwhile a colony of the parasitoid, *Apanteles galleriae* Wilkinson was kept in the laboratory on *Galleria mellonella* (L.) larval stage. To maintain a good parasitoid laboratory population, parasitism was conducted on the early larval instars daily, while adults were feed on pure honey.

2.2. Insecticide:

Lufenuron (Match®) 5% EC, a commercial product of Syngenta AgroSciences Co. was obtained from the local office "SyngentaAgro Egypt", Bldg No.4, 5th floor, Arkan Mall, Sheikh Zayed, 6th of October, office No. 51, Giza, Egypt

2.3. Bioassay procedures:

2.3.1. Wax worm-lufenuron mixed with food procedure:

A series of lufenuron dilutions ranged between 25-250 ppm was prepared in acetone while acetone alone was used for the control. Each dilution was replicated five times; 1 ml of the desirable concentration was mixed with artificial rearing media (5 g) in petri dish (9 cm). The treated media was settled down for one hour then 25 larvae were transferred to each petri dish. The treatments were incubated (21 – 28ºC and 60 – 85% RH). The mortalities were recorded as a percentage of dead larvae after 24, 48 and 72 hours. While for sub-lethal toxicity study, mortality records were continued weekly for three successive weeks. The feeding media was replaced by fresh untreated media every day after 72 h of exposure. Then data were subjected to probit analysis.

2.3.2. Honey bee workers:

Jars (250 ml) with screw caps and small halls were used for bioassay; honey bee workers (n = 10) were isolated in each jar. Lufenuron was dissolved in 10% (w/v) sucrose in water solution to prepare the desirable concentration (500-5000 ppm) and was introduced to the insects in pasteur pipette throw a hall in the screw cap. Concentrations were replicated five times. The jars were settled on 21 – 28º C and 60 – 85% RH and the fresh treated solution was replaced daily. The mortality was recorded as described above.

2.3.3. Parasitoid:

The same insecticide in sucrose solution at the same concentration range which used for honey bee workers was utilized for the parasitid wasp. For every

replicate, 5 pairs of *A. galleriae* adults were transferred from the main colony to small transparent vials (20 ml with screw caps) and was provided with the treated solution through small impregnated cotton pellets and the solution was replaced by fresh treated one daily after 24h of exposure. The mortality was recorded as described above.

2.4. Statistical analysis:

Statistical analyses were performed using the IBM SPSS statistics version 2.0 software package. Before analysis the mortality percentages were transformed into arcsine-square-root and submitted to analysis of variance ANOVA. Means were separated by the Tukey-Kramer honestly significant differences (HDS) at the 5% level (Sokal and Rohlf, 1995). The LC_{50}, LC_{90} and their confidence limits as well as the slopes and their variances were estimated using Ldp Line ® software for probit analysis according to (Finney, 1971).

3.Results

3.1. Susceptibility of *Galleria mellonella* to lufenuron:

The lufenuron concentration–mortality bioassays result against the *G. mellonella* larval instar revealed that, *G. mellonella* larvae showed considerable susceptibility to lufenuron. Moreover, lufenuron toxicity against *G. mellonella* larvae increased in a concentration and time dependent manner with LC_{50} values 87.52, 74.86 and 49.53 ppm after 24, 48 and 72 hours of treatment, respectively Table (1). To investigate the latent toxicity of lufenuron sub-lethal concentrations against *G. mellonella* larvae after 72hours of exposure, concentrations of lufenuron equal to $1/10^{th}$, $1/5^{th}$, half and the LC_{50} values of lufenuron estimated after 72h of exposure, were tested. Data in Fig. (1) showed that, complete mortality of *G. mellonella* larvae was achieved after three weeks following the treatment with lufenuron [49.53 ppm (LC_{50})]. While significantly lower mortality percentages of 61.9, 74.6 and 88.1% were obtained with the lower concentrations $1/10LC_{50}$: 4.95, $1/5LC_{50}$: 9.91, 1/2 LC_{50}: 24.77 ppm, respectively after the same exposure period. Additionally, lufenuron showed significantly high mortality percentages 46.67, 72.67 and 98.46% after three, seven and fifteen days of treatment with LC_{50} compared with the lower concentrations $1/10LC_{50}$ (10.76, 20 and 46.16%), $1/5LC_{50}$ (13.33, 33.33 and 73.39%) and $1/2LC_{50}$ (24, 55.33and 83.15%) after the same period of exposure, respectively. Similarly, the estimated LT_{50} value of lufenuron was significantly low (3.47 day) with the LC_{50} treatment compared with LT_{50} values obtained with $1/10LC_{50}$, $1/5LC_{50}$ and $1/2LC_{50}$ treatments 13.66, 9.64 and 5.80 days, respectively (Table (2)).

3.2. Toxicity of lufenuron against the non-target organisms *Apanteles galleriae* and *Apis mellifera*

Data in Table (3) show that, both insect species *A. galleriae* adults and *A. mellifera* workers showed low susceptibility to lufenuron the LC_{50} values were *A. galleriae* (2695.52 ppm) and *A. mellifera* (2514.38 ppm), after 24 hours of exposure. While, LC_{50} values of lufenuron were (2823.22 and 1769.04ppm) against *A. galleriae*. and (1675.94 and 530.22 ppm) against *A. mellifera*, after 48 and 72 hours of treatment, respectively.

3.3. Selectivity of lufenuron between *G. mellonella* and the non-target organisms *A. galleriae* and *A. mellifera*

Both insect species *A. galleriae* adults and *A. mellifera* workers showed less susceptibility than *G. mellonella* larvae. According to the LC_{50} values the selectivity ratio (SR) between *G. mellonella* and the non-target organisms were calculated as following:

$$SR = \frac{LC_{50} \text{ value obtained for the non-target organism}}{LC_{50} \text{value obtained for } G.\ mellonella} \times 100$$

Table (1): Susceptibility of *G. mellonella* larvae to lufenuron insecticide

Exposure Time (h)	LC_{50} ppm	Confidence Limits at 95% of probability		LC_{90} ppm	Confidence Limits at 95% of probability		Slope ±Variance	X^2
		Lower	Upper		Lower	Upper		
24	87.52	82.98	91.79	276.90	88.56	869.03	3.29 ± 0.88	0.09
48	74.86	67.17	79.07	362.57	90.80	1755.44	2.40 ± 0.53	0.22
72	49.53	40.89	60.03	196.54	86.34	448.52	2.75 ± 0.44	1.05

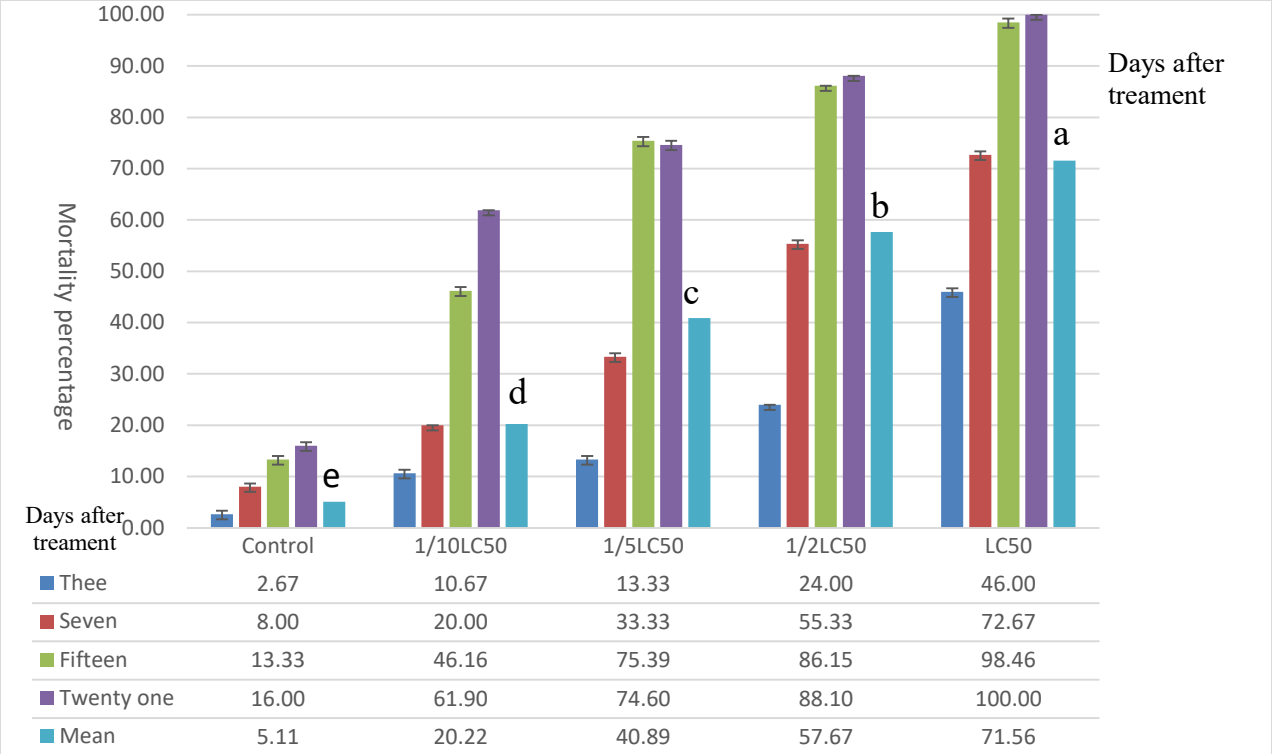

Fig. (1): Effect of sub lethal concentrations of lufenuron on *G. mellonella*

Table (2): Toxicity of sub- lethal concentrations of lufenuron on *G. mellonella* Larvae three weeks after treatment

Concentration ppm	LT_{50} Days	Confidence Limits at 95% of probability		LT_{90} Days	Confidence Limits at 95% of probability		Slope ± Variance	X^2
		Lower	Upper		Lower	Upper		
4.95 (1/10 LC_{50})	13.66	12.00	15.04	52.99	40.57	77.67	2.17 ± 0.21	4.49
9.91 1/5 LC_{50}	9.64	8.38	11.04	33.16	26.86	43.93	2.39 ± 0.21	2.60
24.77 (1/2 LC_{50})	5.80	4.97	6.60	19.33	16.40	24.02	2.45 ± 0.22	2.66
49.53 (LC_{50})	3.47	2.84	4.05	10.02	8.62	12.22	2.79 ± 0.30	5.47

Lufenuron showed selectivity towards *A. galleriae* and *A. mellifera* compared with *G. mellonella* with selectivity ratios 30.80 and 28.73, respectively after 24 hours of treatment, respectively (Fig.2). Furthermore, the selectivity of lufenuron followed the same trend after 48 and 72 hours of exposure as the SR between *A. galleriae* and *G. mellonella* increased 37.71 and 36.26, respectively. However, the SR between *A. mellifera* and *G. mellonella* were gradually decreased from 22.39 after 48 hours of treatment to 10.71 at the end of 72 hours of treatment, respectively.

4.Discussion

Results of the present study demonstrated that, lufenuron was valuable toxic for *G. mellonella* larvae and its toxicity was both concentration and time dependent. Moreover, lufenuron displayed selectivity between *G. mellonella* and the beneficial species *(A. galleriae* adults and *A. mellifera* workers). Furthermore, the selectivity ratio between *G. mellonella and A. galleriae* was rarely changed during three days after exposure. This is in agreement with previous suggestion of little or no injures to adult honey bees caused by IGRs **(Engels, 1990)**. In concordance with previous studies lufenuron assumed to

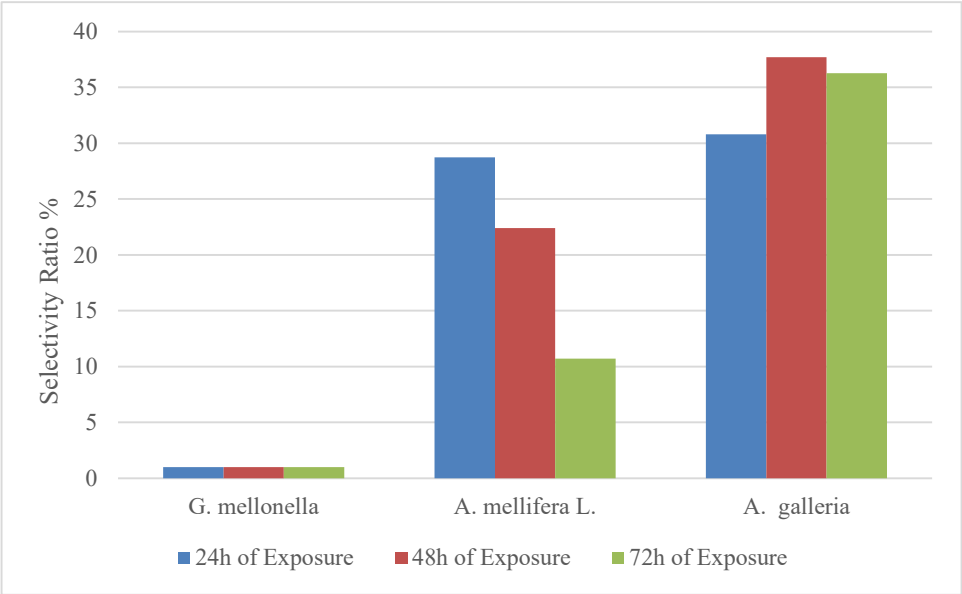

Fig. (2): Selectivity of lufenuron between *G. mellonella* and the non-target organisms *A. galleriae* and *A. mellifera* are presented

be safe for honeybee since no acute or residual toxicity was recorded against *A. mellifera* **(Ahn *et al.*, 2013)**. Also, the results of the current study are comparable with earlier research of **(Duta *et al.*, 2016)** where they assessed the toxicity of four insecticides in use to control mustard aphid (*Lipaphis erysimi* Kilt) against foraging honey bee. They found that, the population in the lufenuron treated plot was slightly decreased (1.42%) compared with the population in the untreated plot at seven days of spray. Therefore, they suggested lufenuron as a safe treatment to foraging honey bee.

Furthermore, in this study low susceptibility of *A. galleriae* to lufenuron was observed; thus, it could be employed effectively in combination with *A. galleriae* to prevent and or control the great wax worm either inside or outside the hive. On account of the importance of *A. galleriae* in supressing the greater wax worm population that reported by many researchers **(Gamal El-Din, 1985 and Mansour & Metwally, 2004 and Hegazy *et al.*, 2019)**. Moreover, according to a previous survey for the natural enemies of the greater wax worm during three years was carried out by **Hanumanthaswamy and Rajagopal (2017)**, they found that, *A. galleriae* was recognized as the master species amongst other recorded natural enemies. Earlier study indicated that, *A. galleriae* was parasitized 15% of *G. mellonella* larvae **(Shimamori, 1987)**. Additionally, treatment of *G. mellonella* larvae with *A. galleriae* caused 59.5% reduction of larvae numbers within 10 days after treatment **(Mansour *et al.*, 2010)**. Therefore, *A. galleriae* could be employed with other methods to control *G. mellonella*. In the meantime, regarding of exposure to the sublethal concentrations of lufenuron, even though all tested concentrations displayed no effects against *A. galleriae* and *A. mellifera,* The LC_{50} could be the best suggestion concentration to use against *G. mellonella*; on account of that lufenuron at this concentration provided complete suppression of *G. mellonella* without any toxicity to both the *A. galleriae*. However, 1/5 LC_{50} and 1/2 LC_{50} of lufenuron may be suitable concentrations to combined with the biological control. As these low concentrations confer opportunity for efficient parasitism of A. *galleria*. Furthermore, lufenuron could be consider environmentally friendly insecticide for *G. mellonella*, hence it inhibits chitin synthesis and disrupt the development during molting, attributable to its lipophilic affinity it interferes with the exoskeleton by contact, in addition it acts as antifeedent **(Engels, 1990)**. Among pest control methods integrated pest management (IPM) is considered the sensible measures to provide effective protection and control against pest infestations. In this regard, employing of lufenuron in combination with the effective parasitoid *A. galleria* could be serve in IPM program to control *G. mellonella* inside and outside hives. However, more advanced research may be required to study the biological and biochemical effects of lufenuron on the three tested species.

Table (3): Toxicity of lufenuron on the none target organisms *A. mellifera*, and *A. galleria* adults

Exposure Time (h)	Insect species	LC_{50} ppm	Confidence Limits at 95% of probability		LC_{90} ppm	Confidence Limits at 95% of probability		Slope ±Variance	X^2
			Lower	Upper		Lower	Upper		
24	*A. mellifera*	2514.38	1977.77	3945.43	12599.7	6704.34	47105.71	1.83 ± 0.34	0.04
	A. galleria	2695.52	2461.57	2957.31	8026.25	6864.10	9751.92	2.71 ± 0.18	0.02
48	*A. mellifera*	1675.94	1433.57	2096.30	7082.76	4597.62	15595.41	2.05 ±0.35	5.62
	A. galleria	2823.22	2512.89	3254.79	10227.55	15423.27	7712.76	2.29 ± 0.23	0.0006
72	*A. mellifera*	530.23	351.06	669.95	3016.51	2201.14	5497.36	1.70 ± 0.30	4.94
	A. galleria	1796.04	1576.38	2032.55	8394.56	6770.79	11089.44	1.91 ± 0.15	1.84

Conclusion

The effects of lethal and sublethal concentration of lufenuron against *G. mellonella* comparative with its effects on *A. galleria* and *A. mellifera.* suggested that, lufenuron provided adequate suppression of *G. mellonella* with no adverse toxic effects on both *A. galleria* and *A. mellifera*. Consequently, lufenuron could be utilized as a relatively specific insecticide to control *G. mellonella*. Moreover, the binary effect of sublethal concentrations of lufenuron with the parasitoid *A. galleria* may be employed effectively in IPM program to control G. *mellonella.* The present study highlights the dual effects of biological control and IGRs as an accessible approach to *G. mellonella* control exclusively throughout the periods of bee colonies weakness.

Acknowledgements

Sincere appreciation is going to Professor S. M. Abd-ElRahman, Central Agricultural Pesticides Lab. **(**CAPL), Agriculture Research, for her continuous help and useful discussion during the course of this study. As well as for the all through the manuscript preparation.

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تقييم سمية مبيد لوفينورون ومقارنتها ضد كل من دودة الشمع الكبرى وطفيل ابنتليس جيليااريا وشغالات النحل

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الملخص العربى:

تعد دودة الشمع الكبرى من اكثر أفات النحل خطورة فى مصر والعالم أجمع. طرق مكافحة هذه الآفة محدودة حيث أن كثير من الاعتبارات البيئية والصحية يجب مراعاتها عند مكافحتها. كما يعد طفيل ابنتليس جيليااريا من اهم اعداء دودة الشمع فى العالم ومتواجد فى مصر ايضا. ومن ناحية اخرى المبيد الحشرى لوفينورون (مانع انسلاخ) مثبت علميا تأثيره ضد أفات عائلة لبيدوبترا كما انه ليس له اثار ضارة على النحل. لذلك كان هدف هذه الدراسة مقارنة سمية مبيد ليفينيرون ضد كل من دودة الشمع الكبرى و طفيل الابنتليس جيليااريا والنحل. لاتمام هذه الدراسة تم التققيم الحيوى بطريقة الخلط مع البيئة الغذائية لتقدير سمية مبيد اليفينيرون لكل من يرقات دودة الشمع والحشرات الكاملة من الطفيل بالاضافة الى شغلات نحل العسل. أظهرت النتائج اختيارية مبيد لوفينورون لدودة الشمع فقد كانت قيم الجرعات المميته ل ٥٠% من الافراد المعاملة كالاتى: ٨٧,٥٢, ٧٤,٨٦, ٤٩,٥٣ جزء فى المليون بعد ٢٤, ٤٨, ٧٢ ايام من المعاملة على التوالى. فى المقابل كانت حساسية كل من الطفيل والنحل لمبيد اللوفينورون منخفضة حيث كانت قيم الجرعات المميته ل ٥٠% من الافراد المعاملة كالاتى: ٢٥١٤,٥٢ و ٢٦٩٥,٥٢ جزء فى المليون بعد ٢٤ ساعة على التوالى. وايضا كانت قيم نسبة الاختيارية قياسا على قيم الجرعات المميته ل ٥٠% من الافراد المعاملة منسوبة لنفس القيمة الخاصة بدودة الشمع ٣٠,٨٠ ضعف للنحل و ٢٨,٧٣ ضعف للطفيل بعد ٢٤ ساعة من المعاملة. علاوة على ذلك لم تؤدى المعاملة بالجرعات التحت مميته من مبيد اللوفينورون اى سمية لكل من الطفيل والنحل حتى ثلاث اسابيع بعد المعاملة. على النقيض كانت نسب موت دودة الشمع ٧٢,٦٧, ٩٨,٤٦, ١٠٠ % نتيجة المعاملة بالجرعة المميته ل ٥٠% من الافراد المعاملة (٤٩,٥٣) جزء فى المليون بعد ١, ٢, ٣ اسابيع من المعاملة. بناء على نتائج هذه الدراسة يمكن التوصية بتوظيف مبيد لوفينورون لمكافحة دودة الشمع الكبرى كمبيد متخصص الى حد كبير وغير ضار لكل من العدو الطبيعى لدودة الشمع او النحل. هذه الدراسة تلقى الضوء على التأثير الثنائى للمكافحة الحيوية و منظمات النمو الحشرية كعنصر واعد فى مكافحة دودة الشمع الكبرى خاصة فى فترات ضعف الخليه.